



## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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<b>(21) International Application Number:</b> PCT/AU00/00424 <b>(22) International Filing Date:</b> 8 May 2000 (08.05.00)  <b>(30) Priority Data:</b> PQ 0219                      7 May 1999 (07.05.99)                      AU PQ 0220                      7 May 1999 (07.05.99)                      AU  <b>(71) Applicant (for all designated States except US):</b> BIOFAN PTY LTD [AU/AU]; 92 Station Street, Carlton, Victoria 3053 (AU).  <b>(72) Inventor; and</b> <b>(75) Inventor/Applicant (for US only):</b> HARRIS, George [AU/AU]; 92 Station Street, Carlton, Victoria 3053 (AU).  <b>(74) Agents:</b> SLATTERY, John, M. et al.; Davies Collison Cave, 1 Little Collins Street, Melbourne, Victoria 3000 (AU).		<b>(81) Designated States:</b> AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).  <b>Published</b> <i>With international search report.</i>
<b>(54) Title:</b> A METHOD OF PROPHYLAXIS AND TREATMENT AND AGENTS USEFUL THEREFOR		
<b>(57) Abstract</b> <p>The present invention relates generally to a method of down-regulating or otherwise reducing the functional levels of an endogenously produced molecule in a subject and agents useful for same. More particularly, the present invention relates to a method of reducing the functional levels of one or more complement components, a cytokine or an adhesion molecule in a subject and agents useful for same. Still more particularly, the present invention contemplates a method of down-regulating or otherwise reducing the functional levels of a cytokine by administering to said subject a cytokine and/or cytokine receptor, or a derivative, homologue, analogue, chemical equivalent thereof, immunogenic composition. The method of the present invention is useful, <i>inter alia</i>, in a range of therapeutic and prophylactic applications.</p>		

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## A METHOD OF PROPHYLAXIS AND TREATMENT AND AGENTS USEFUL THEREFOR

### FIELD OF THE INVENTION

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The present invention relates generally to a method of down-regulating or otherwise reducing the functional levels of an endogenously produced molecule in a subject and agents useful for same. More particularly, the present invention relates to a method of reducing the functional levels of one or more complement components, a cytokine or an  
10 adhesion molecule in a subject and agents useful for same. Still more particularly, the present invention contemplates a method of down-regulating or otherwise reducing the functional levels of a cytokine by administering to said subject a cytokine and/or cytokine receptor, or a derivative, homologue, analogue, chemical equivalent thereof, immunogenic composition. The method of the present invention is useful, *inter alia*, in a range of  
15 therapeutic and prophylactic applications.

### BACKGROUND OF THE INVENTION

Bibliographic details of the publications referred to by author in this specification are  
20 collected at the end of the description.

Cytokines are pleiotropic molecules which, *inter alia*, impart signals to cells to regulate the immune response, control cell proliferation and differentiation and to regulate the operation of the cytokine network. However, in addition to their beneficial effect,  
25 cytokines also induce adverse side effects such as inhibition of normal cell growth, undesirable modulation of the functional activity of other cytokines or other unwanted immune effects such as severe inflammation, fever, malaise, nausea or leukopenia. For example, chronic inflammation is facilitated by the presence of GM-CSF and/or M-CSF and/or TNF- $\alpha$ .

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To date methods for therapeutically or prophylactically reducing unwanted cytokine effects have centred on the administration of antibodies directed to cytokines which have been raised in mice. Alternatively, the parenteral administration of cloned soluble receptors has been utilised both therapeutically and prophylactically. However, repeated dosing of  
5 murine proteins, such as antibodies, leads to the development of anti-murine antibodies thereby causing both rapid clearance of the murine antibodies and the production of immune complexes which cause the HAMA response.

Accordingly, a need exists to develop methods of reducing levels of unwanted  
10 endogenously produced molecules such as cytokines in a subject in a specific manner and without the risk of inducing further immune complications of the type related to the HAMA response.

In work leading up to the present invention, the inventors have developed a method of  
15 reducing the level of unwanted endogenously produced molecules by inducing, in the subject to be treated, an immune response specifically directed to said molecule.

#### SUMMARY OF THE INVENTION

20 Throughout this specification and the claims which follow, unless the context requires otherwise, the word "comprise", and variations such as "comprises" and "comprising", will be understood to imply the inclusion of a stated integer or step or group of integers or steps but not the exclusion of any other integer or step or group of integers or steps.

25 The subject specification contains amino acid sequence information prepared using the programme PatentIn Version 2.0, presented herein after the bibliography. Each amino acid sequence is identified in the sequence listing by the numeric indicator <210> followed by the sequence identifier (e.g. <210> 1, <210> 2, etc). The length, type of sequence (protein (PRT), etc) and source organism for each amino acid sequence is  
30 indicated by information provided in the numeric indicator fields <211>, <212> and <213>, respectively. Amino acid sequences referred to in the specification are defined

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by the information provided in numeric indicator field <400> followed by the sequence identifier (e.g. <400> 1, <400> 2, etc).

One aspect of the present invention provides a method of down regulating or otherwise  
5 reducing the functional level of an endogenously-produced molecule in a subject said  
method comprising administering to said subject an effective amount of an agent, the agent  
comprising said molecule or the ligand of said molecule or a derivative, homologue,  
analogue, chemical equivalent or mimetic thereof for a time and under conditions  
sufficient to induce, upregulate or otherwise elicit an immune response directed to said  
10 molecule and/or said ligand.

Another aspect of the present invention provides a method of down-regulating or otherwise  
reducing the functional level of a complement component in a subject said method  
comprising administering to said subject an effective amount of an agent, the agent  
15 comprising said complement component or the ligand of said complement component or a  
derivative, homologue, analogue, chemical equivalent or mimetic thereof for a time and  
under conditions sufficient to induce, upregulate or otherwise elicit an immune response  
directed to said complement component and/or said ligand.

20 Still another aspect of the present invention provides a method of down-regulating or  
otherwise reducing the functional level of a cytokine in a subject said method comprising  
administering to said subject an effective amount of an agent, the agent comprising said  
cytokine and/or said cytokine receptor or a derivative, homologue, analogue, chemical  
equivalent or mimetic thereof for a time and under conditions sufficient to induce, up-  
25 regulate or otherwise elicit an immune response directed to said cytokine and/or said  
cytokine receptor.

Still yet another aspect of the present invention provides a method of down-regulating or  
otherwise reducing the functional level of GM-CSF in a subject said method comprising  
30 administered to said subject an effective amount of an agent, the agent comprising GM-  
CSF and/or GM-CSF receptor or a derivative, homologue, analogue, chemical equivalent

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or mimetic thereof for a time and under conditions sufficient to induce, up-regulate or otherwise elicit an immune response directed to GM-CSF and/or GM-CSF receptor.

Yet another aspect of the present invention provides a method of down-regulating or  
5 otherwise reducing the functional level of M-CSF in a subject said method comprising administering to said subject an effective amount of an agent, the agent comprising M-CSF and/or M-CSF receptor or a derivative, homologue, analogue, chemical equivalent or mimetic thereof for a time and under conditions sufficient to induce, up-regulate or otherwise elicit an immune response directed to said M-CSF and/or M-CSF receptor.

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A further aspect of the present invention provides a method of down-regulating or otherwise reducing the functional level of an adhesion molecule in a subject said method comprising administering to said subject an effective amount of an agent, the agent comprising said adhesion molecule and/or adhesion molecule ligand or a derivative,  
15 homologue, analogue, chemical equivalent or mimetic thereof for a time and under conditions sufficient to induce, up-regulate or otherwise elicit an immune response directed to said adhesion molecule and/or adhesion molecule ligand.

Another further aspect of the present invention provides a method of down-regulating or  
20 otherwise reducing the functional level of TNF- $\alpha$  in a subject said method comprising administering to said subject an effective amount of an agent, the agent comprising TNF- $\alpha$  and/or TNF- $\alpha$  receptor or a derivative, homologue, analogue, chemical equivalent or mimetic thereof for a time and under conditions sufficient to induce, up-regulate or otherwise elicit an immune response directed to said TNF- $\alpha$  and/or TNF- $\alpha$  receptor.

25

Yet another further aspect of the present invention provides a method of down-regulating or otherwise reducing the functional level of Integrin  $\alpha_4 \beta_1$  in a subject said method comprising administering to said subject an effective amount of an agent comprising said Integrin  $\alpha_4 \beta_1$  and/or VCAM-1 and/or fibronectin or a derivative, homologue, analogue,  
30 chemical equivalent or mimetic thereof for a time and under conditions sufficient to induce, up-regulate or otherwise elicit and immune response directed to said Integrin  $\alpha_4 \beta_1$

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and/or said VCAM-1 and/or fibronectin.

Still yet another further aspect of the present invention provides a method for the treatment or prophylaxis of a disease condition involving unwanted endogenously produced molecule  
5 functional activity in a subject said method comprising administering to said subject an effective amount of an agent, the agent comprising said molecule and/or molecule ligand or a derivative, homologue, analogue, chemical equivalent or mimetic thereof for a time and under conditions sufficient to induce, up-regulate or otherwise elicit an immune response directed to said molecule and/or molecule ligand wherein the functional level of  
10 said molecule is down-regulated.

In a further aspect the present invention provides an immunogenic agent for use in inducing, up-regulating or otherwise eliciting an immune response to an endogenously-produced molecule or ligand thereof, said composition comprising at least one B cell  
15 epitope linked, bound or otherwise associated with at least one T cell epitope.

The present invention also provides an immunogenic agent for use in inducing, up-regulating or otherwise eliciting an immune response to an endogenously-produced molecule or ligand thereof said composition comprising at least two B cell epitopes which  
20 epitopes are each linked, bound or otherwise associated with at least three T cell epitopes.

Yet another aspect of the present invention provides a pharmaceutical composition for use in down-regulating the functional activity of an endogenously-produced molecule comprising an agent as hereinbefore defined together with any one or more  
25 pharmaceutically acceptable carriers and/or diluents. These components are referred to as the active ingredients.

Yet another aspect of the present invention is directed to an immunogenic agent as hereinbefore defined when used in accordance with the method of the present invention.  
30

Still another aspect of the present invention provides an agent useful for down-regulating

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the functional level of an endogenously-produced molecule in a subject as hereinbefore defined.

Still yet another aspect of the present invention provides an immunogenic agent in the  
5 manufacture of a medicament for the treatment of a disease condition characterised by  
unwanted endogenously-produced molecule levels.



**DETAILED DESCRIPTION OF THE INVENTION**

The present invention is predicated, in part, on the development of a method for reducing the functional levels of one or more endogenously produced molecules in an individual by  
5 eliciting, in said individual, autoantibodies to the molecule or its ligand. This has facilitated the development of controlled methods for therapeutically and/or prophylactically treating disease conditions by selectively reducing functional levels of one or more specific molecules such as complement components, cytokines or adhesion molecules.

10

Accordingly, one aspect of the present invention provides a method of down regulating or otherwise reducing the functional level of an endogenously-produced molecule in a subject said method comprising administering to said subject an effective amount of an agent, the agent comprising said molecule or the ligand of said molecule or a derivative, homologue,  
15 analogue, chemical equivalent or mimetic thereof for a time and under conditions sufficient to induce, upregulate or otherwise elicit an immune response directed to said molecule and/or said ligand.

Reference to an "endogenously-produced molecule" should be understood as a reference to  
20 any molecule which is produced, *in vivo*, by a subject. The molecule may be constitutively produced or may be produced as a result of a specific stimulus. It should be understood that the molecule may be soluble (such as, but not limited to, complement components or cytokines) or the molecule may be bound to a structure such as a cell membrane or extracellular matrix (for example, adhesion molecules). Reference to the  
25 "ligand" of an endogenously produced molecule should be understood as a reference to any other molecule to which the endogenously produced molecule binds or otherwise associates. For example, where the endogenously produced molecule is a cytokine, the cytokine receptor is a ligand. In another example, where the endogenously produced molecule is C1, the antigen/antibody complex to which it binds is a ligand, or where the  
30 endogenously produced molecule is C2, the ligand may be C4b.

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Preferably, the endogenously produced molecule is a complement component, cytokine or adhesion molecule.

Accordingly, the present invention provides a method of down-regulating or otherwise  
5 reducing the functional level of a complement component in a subject said method  
comprising administering to said subject an effective amount of an agent, the agent  
comprising said complement component or the ligand of said complement component or a  
derivative, homologue, analogue, chemical equivalent or mimetic thereof for a time and  
under conditions sufficient to induce, upregulate or otherwise elicit an immune response  
10 directed to said complement component and/or said ligand.

Reference to "complement component" should be understood as a reference to any  
component of the alternative or classical complement pathway or the cleavage product of  
said component or the complement complexes which are formed at the various stages of  
15 the complement cascade. Examples of complement components include, but is not limited  
to, C1q, C1r, C1s, C3, C4, Factor D, plasma protease, Factor B, the cleavage products  
C2b, C2a, C3b, or the complexes C3bBb, C4b2b or the membrane attack complex.

In another embodiment the present invention provides a method of down-regulating or  
20 otherwise reducing the functional level of a cytokine in a subject said method comprising  
administering to said subject an effective amount of an agent, the agent comprising said  
cytokine and/or said cytokine receptor or a derivative, homologue, analogue, chemical  
equivalent or mimetic thereof for a time and under conditions sufficient to induce, up-  
regulate or otherwise elicit an immune response directed to said cytokine or said cytokine  
25 receptor.

Reference to "agent" should be understood as a reference to the composition which is  
administered to the subject in accordance with the method of the present invention. The  
agent may comprise the immunogenic molecule alone or it may also comprise other  
30 proteinaceous or non-proteinaceous molecules such as, but not limited to, a T cell  
epitope, carrier molecule or adjuvant. The agent may therefore take the form of, for

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example, a purified cytokine or a derivative, homologue, analogue, chemical equivalent or mimetic thereof or a pharmaceutical formulation, such as a vaccine formulation, which comprises for example, said purified cytokine or derivative, homologue, analogue, chemical equivalent or mimetic thereof alone or together with another proteinaceous or  
5 non-proteinaceous molecule such as a T cell epitope, carrier molecule or adjuvant.

Reference to "subject" should be understood as a reference to any animal or bird such as but not limited to a human, primate, livestock animal (e.g. sheep, cow, horse, donkey, pig), companion animal (e.g. dog, cat), laboratory test animal (e.g. mouse, rabbit, rat,  
10 guinea pig, hamster), captive wild animal (e.g. fox, deer), caged bird (e.g. parrot) and poultry bird (e.g. chicken, duck, pheasant, turkey. Preferably, the subject is a human or primate. Most preferably, the subject is a human.

Reference to the "functional level" of an endogenously produced molecule should be  
15 understood as a reference to the level of molecule, in a subject, which is able to fulfil its biological role of binding to a ligand thereby contributing to a biological effect. The "functional level" of a molecule may be down-regulated by any one of a number of mechanisms including, but not limited to:

- 20 (i) Reducing the actual levels of the molecule thereby reducing the amount of molecule available to bind to its ligand. This may be achieved, for example, by capturing a molecule with an antibody, which molecule-antibody complex is then removed by any one of a number of effector mechanisms such as via the actions of a macrophage or functionally similar cell type.
- 25 (ii) Blocking the ligand binding portion of a molecule with an antibody. Although the actual levels of a given molecule are not reduced, the levels of molecule with a free ligand binding portion are reduced. Accordingly, the overall level of molecule available to interact with its ligand is down-regulated.
- 30 (iii) Blocking the molecule's ligand utilising an antibody directed to the molecule

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binding site. Although overall levels of the molecule are not reduced, the availability of a ligand to which these molecules can bind is reduced.

Reference to a "cytokine" should be understood as a reference to a protein hormone. The  
5 cytokine may be in a soluble form or may be anchored to a surface such as, but not limited to, a cell membrane or an extracellular matrix surface. Even where a cytokine is anchored, for example to an extracellular matrix, the cytokine may still exhibit functional activity by binding to localised cells expressing the receptor for that cytokine. In this regard, it may therefore also be desirable to reduce the capacity of these cytokines to bind  
10 to a target.

Particularly preferred cytokines are those which are unwanted such as those which are associated with any one or more symptoms of a disease condition. Reduction of the functional levels of those cytokines thereby reduces or ameliorates any one or more  
15 symptoms associated with such a disease condition. For example, GM-CSF, M-CSF, TNF $\alpha$ , IL-1 and/or IL-6 are present in elevated levels in rheumatoid arthritis, Crohn's disease, Type I diabetes, multiple sclerosis, psoriasis and chronic inflammatory lung disease (for example, asthma, chronic bronchitis, emphysema, chronic obstructive airway disease). IL-4 and IL-5 are active in allergic lung diseases such as asthma,  
20 bronchopulmonary aspergillosis, allergic granulomatous disease, anaphylactic reactions and parasitaemia. Interferon- $\gamma$ , IL-12 and TNF $\alpha$  are functionally active in infectious and non-infectious lung disease such as chronic bronchitis and cystic fibrosis while TGF $\beta$  is produced during formation of lung fibrosis. The onset of inflammatory bowel disease involves the production of GM-CSF, M-CSF, TNF $\alpha$  and IL-1 while interferon- $\gamma$  and  
25 interferon- $\alpha$  are produced in an individual suffering from Type I diabetes or multiple sclerosis. GM-CSF and M-CSF are also involved in atherosclerosis, osteoporosis, myeloid leukaemia and solid tumours. Angiogenesis is known to involve the production of VEGF, FGF and FGF/CTGF.

30 Preferably, said cytokines are GM-CSF, M-CSF and TNF- $\alpha$ .

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Accordingly, in a preferred embodiment the present invention provides a method of down-regulating or otherwise reducing the functional level of GM-CSF in a subject said method comprising administered to said subject an effective amount of an agent, the agent comprising GM-CSF and/or GM-CSF receptor or a derivative, homologue, analogue,  
5 chemical equivalent or mimetic thereof for a time and under conditions sufficient to induce, up-regulate or otherwise elicit an immune response directed to GM-CSF and/or GM-CSF receptor.

In yet another preferred embodiment the present invention provides a method of down-regulating or otherwise reducing the functional level of M-CSF in a subject said method  
10 comprising administering to said subject an effective amount of an agent, the agent comprising M-CSF and/or M-CSF receptor or a derivative, homologue, analogue, chemical equivalent or mimetic thereof for a time and under conditions sufficient to induce, up-regulate or otherwise elicit an immune response directed to said M-CSF and/or  
15 M-CSF receptor.

In still yet another preferred embodiment the present invention provides a method of down-regulating or otherwise reducing the functional level of TNF- $\alpha$  in a subject said method comprising administering to said subject an effective amount of an agent, the agent  
20 comprising TNF- $\alpha$  and/or TNF- $\alpha$  receptor or a derivative, homologue, analogue, chemical equivalent or mimetic thereof for a time and under conditions sufficient to induce, up-regulate or otherwise elicit an immune response directed to said TNF- $\alpha$  and/or TNF- $\alpha$  receptor.

25 In another embodiment of the present invention, instead of, or in addition to down-regulating functional levels of one or more cytokines, it may also be desirable to down-regulate functional levels of adhesion molecules. Adhesion molecules are involved in, *inter alia*, leukocyte migration across tissues (such across the vascular endothelium), homing and cell-cell interactions. By reducing the availability of either the adhesion  
30 molecule or its ligand, the modulation of leukocyte homing, interactions and migration can be achieved. In this regard, it should be understood that the term "functional levels" has

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the same meaning with respect to adhesion molecule ligands and adhesion molecules as it does with respect to cytokines and cytokine receptors, respectively.

Accordingly, another embodiment of the present invention provides a method of down-regulating or otherwise reducing the functional level of an adhesion molecule in a subject  
5 said method comprising administering to said subject an effective amount of an agent, the agent comprising said adhesion molecule and/or adhesion molecule ligand or a derivative, homologue, analogue, chemical equivalent or mimetic thereof for a time and under conditions sufficient to induce, up-regulate or otherwise elicit an immune response  
10 directed to said adhesion molecule and/or adhesion molecule ligand.

Reference to "adhesion molecule" should be understood as a reference to any molecule, the functional activity of which includes, facilitating leukocyte migration, homing or interaction. These molecules are generally anchored to a cell membrane or extracellular  
15 matrix. Adhesion molecules can be broadly grouped as selectins, mucin-like vascular addressins, integrins and the adhesion molecules of the immunoglobulin super family. Particularly preferred adhesion molecules are the integrins such as, but not limited to, Integrin  $\alpha_4 \beta_1$  which is bound by VCAM-1 and fibronectin (CS-1). Integrin  $\alpha_4 \beta_1$  is also known as VLA-4, LPAM-1, CD49d/CD29. Without limiting the invention in any way,  
20 the integrins comprise an  $\alpha$  and  $\beta$  chain.

Accordingly, in a preferred aspect the present invention provides a method of down-regulating or otherwise reducing the functional level of Integrin  $\alpha_4 \beta_1$  in a subject said method comprising administering to said subject an effective amount of an agent  
25 comprising said Integrin  $\alpha_4 \beta_1$  and/or VCAM-1 and/or fibronectin or a derivative, homologue, analogue, chemical equivalent or mimetic thereof for a time and under conditions sufficient to induce, up-regulate or otherwise elicit an immune response directed to said Integrin  $\alpha_4 \beta_1$  and/or said VCAM-1 and/or fibronectin.

30 "Derivatives" should be understood to include fragments, parts, portions, mutants, cyclised peptides, and mimetics from natural, synthetic or recombinant sources including

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fusion proteins. Fragments and parts may be epitope regions, for example. Derivatives may be derived from insertion, deletion or substitution of amino acids. Amino acid insertional derivatives include amino and/or carboxylic terminal fusions as well as intrasequence insertions of single or multiple amino acids. Insertional amino acid

5 sequence variants are those in which one or more amino acid residues are introduced into a predetermined site in the protein although random insertion is also possible with suitable screening of the resulting product. Deletional variants are characterized by the removal of one or more amino acids from the sequence. Substitutional amino acid variants are those in which at least one residue in the sequence has been removed and a different residue

10 inserted in its place. An example of substitutional amino acid variants are conservative amino acid substitutions. Conservative amino acid substitutions typically include substitutions within the following groups: glycine and alanine; valine, isoleucine and leucine; aspartic acid and glutamic acid; asparagine and glutamine; serine and threonine; lysine and arginine; and phenylalanine and tyrosine. Additions to amino acid sequences

15 including fusions with other peptides, polypeptides or proteins.

Homologues of the protein contemplated herein include, but are not limited to, proteins derived from different species.

20 Chemical and functional equivalents should be understood as molecules exhibiting any one or more of the functional activities and may be derived from any source such as being chemically synthesized or identified via screening processes such as natural product screening.

25 The derivatives include fragments having particular epitopes of parts of the entire protein fused to peptides, polypeptides or other proteinaceous or non-proteinaceous molecules.

Analogues contemplated herein include, but are not limited to, modification to side chains, incorporating of unnatural amino acids and/or their derivatives during peptide, polypeptide

30 or protein synthesis and the use of crosslinkers and other methods which impose conformational constraints on the proteinaceous molecules or their analogs.

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Derivatives of nucleic acid sequences may similarly be derived from single or multiple nucleotide substitutions, deletions and/or additions including fusion with other nucleic acid molecules. The derivatives of the nucleic acid molecules of the present invention include oligonucleotides, PCR primers, antisense molecules, molecules suitable for use in  
5 cosuppression and fusion of nucleic acid molecules. Derivatives of nucleic acid sequences also include degenerate variants.

Examples of side chain modifications contemplated by the present invention include modifications of amino groups such as by reductive alkylation by reaction with an  
10 aldehyde followed by reduction with  $\text{NaBH}_4$ ; amidination with methylacetimidate; acylation with acetic anhydride; carbamoylation of amino groups with cyanate; trinitrobenzylation of amino groups with 2, 4, 6-trinitrobenzene sulphonic acid (TNBS); acylation of amino groups with succinic anhydride and tetrahydrophthalic anhydride; and pyridoxylation of lysine with pyridoxal-5-phosphate followed by reduction with  $\text{NaBH}_4$ .  
15

The guanidine group of arginine residues may be modified by the formation of heterocyclic condensation products with reagents such as 2,3-butanedione, phenylglyoxal and glyoxal.

20 The carboxyl group may be modified by carbodiimide activation *via* O-acylisourea formation followed by subsequent derivitisation, for example, to a corresponding amide.

Sulphydryl groups may be modified by methods such as carboxymethylation with iodoacetic acid or iodoacetamide; performic acid oxidation to cysteic acid; formation of a  
25 mixed disulphides with other thiol compounds; reaction with maleimide, maleic anhydride or other substituted maleimide; formation of mercurial derivatives using 4-chloromercuribenzoate, 4-chloromercuriphenylsulphonic acid, phenylmercury chloride, 2-chloromercuri-4-nitrophenol and other mercurials; carbamoylation with cyanate at alkaline pH.



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Tryptophan residues may be modified by, for example, oxidation with N-bromosuccinimide or alkylation of the indole ring with 2-hydroxy-5-nitrobenzyl bromide or sulphenyl halides. Tyrosine residues on the other hand, may be altered by nitration with tetranitromethane to form a 3-nitrotyrosine derivative.

5

Modification of the imidazole ring of a histidine residue may be accomplished by alkylation with iodoacetic acid derivatives or N-carboethoxylation with diethylpyrocarbonate.

10 Examples of incorporating unnatural amino acids and derivatives during protein synthesis include, but are not limited to, use of norleucine, 4-amino butyric acid, 4-amino-3-hydroxy-5-phenylpentanoic acid, 6-aminohexanoic acid, t-butylglycine, norvaline, phenylglycine, ornithine, sarcosine, 4-amino-3-hydroxy-6-methylheptanoic acid, 2-thienyl alanine and/or D-isomers of amino acids. A list of unnatural amino acid contemplated  
15 herein is shown in Table 1.

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TABLE 1

	Non-conventional amino acid	Code	Non-conventional amino acid	Code
5				
	$\alpha$ -aminobutyric acid	Abu	L-N-methylalanine	Nmala
	$\alpha$ -amino- $\alpha$ -methylbutyrate	Mgab	L-N-methylarginine	Nmarg
	aminocyclopropane- carboxylate	Cpro	L-N-methylasparagine	Nmasn
			L-N-methylaspartic acid	Nmasp
10	aminoisobutyric acid	Aib	L-N-methylcysteine	Nmcys
	aminonorbornyl- carboxylate	Norb	L-N-methylglutamine	Nmgln
			L-N-methylglutamic acid	Nmglu
	cyclohexylalanine		Chexa L-N-methylhistidine	Nmhis
	cyclopentylalanine	Cpen	L-N-methylisoleucine	Nmile
15	D-alanine	Dal	L-N-methylleucine	Nmleu
	D-arginine	Darg	L-N-methyllysine	Nmlys
	D-aspartic acid	Das	L-N-methylmethionine	Nmmet
	D-cysteine	Dcys	L-N-methylnorleucine	Nmnle
	D-glutamine	Dgln	L-N-methylnorvaline	Nmnva
20	D-glutamic acid	Dglu	L-N-methylornithine	Nmorn
	D-histidine	Dhis	L-N-methylphenylalanine	Nmphe
	D-isoleucine	Dile	L-N-methylproline	Nmpro
	D-leucine	Dleu	L-N-methylserine	Nmser
	D-lysine	Dlys	L-N-methylthreonine	Nmthr
25	D-methionine	Dmet	L-N-methyltryptophan	Nmtrp
	D-ornithine	Dorn	L-N-methyltyrosine	Nmtyr
	D-phenylalanine	Dphe	L-N-methylvaline	Nmval
	D-proline	Dpro	L-N-methylethylglycine	Nmetg
	D-serine	Dser	L-N-methyl-t-butylglycine	Nmtbug
30	D-threonine	Dthr	L-norleucine	Nle
	D-tryptophan	Dtrp	L-norvaline	Nva

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	D-tyrosine	Dtyr	$\alpha$ -methyl-aminoisobutyrate	Maib
	D-valine	Dval	$\alpha$ -methyl- $\gamma$ -aminobutyrate	Mgab
	D- $\alpha$ -methylalanine	Dmala	$\alpha$ -methylcyclohexylalanine	Mchexa
	D- $\alpha$ -methylarginine	Dmarg	$\alpha$ -methylcyclopentylalanine	Mcpen
5	D- $\alpha$ -methylasparagine	Dmasn	$\alpha$ -methyl- $\alpha$ -naphthylalanine	Manap
	D- $\alpha$ -methylaspartate	Dmasp	$\alpha$ -methylpenicillamine	Mpen
	D- $\alpha$ -methylcysteine	Dmcys	N-(4-aminobutyl)glycine	Nglu
	D- $\alpha$ -methylglutamine	Dmgln	N-(2-aminoethyl)glycine	Naeg
	D- $\alpha$ -methylhistidine	Dmhis	N-(3-aminopropyl)glycine	Norn
10	D- $\alpha$ -methylisoleucine	Dmile	N-amino- $\alpha$ -methylbutyrate	Nmaabu
	D- $\alpha$ -methylleucine	Dmleu	$\alpha$ -naphthylalanine	Anap
	D- $\alpha$ -methyllysine	Dmlys	N-benzylglycine	Nphe
	D- $\alpha$ -methylmethionine	Dmmet	N-(2-carbamylethyl)glycine	Ngln
	D- $\alpha$ -methylornithine	Dmorn	N-(carbamylmethyl)glycine	Nasn
15	D- $\alpha$ -methylphenylalanine	Dmphe	N-(2-carboxyethyl)glycine	Nglu
	D- $\alpha$ -methylproline	Dmpro	N-(carboxymethyl)glycine	Nasp
	D- $\alpha$ -methylserine	Dmser	N-cyclobutylglycine	Ncbut
	D- $\alpha$ -methylthreonine	Dmthr	N-cycloheptylglycine	Nchep
	D- $\alpha$ -methyltryptophan	Dmtrp	N-cyclohexylglycine	Nchex
20	D- $\alpha$ -methyltyrosine	Dmtty	N-cyclodecylglycine	Ncdec
	D- $\alpha$ -methylvaline	Dmval	N-cylcododecylglycine	Ncdod
	D-N-methylalanine	Dnmala	N-cyclooctylglycine	Ncoct
	D-N-methylarginine	Dnmarg	N-cyclopropylglycine	Ncpro
	D-N-methylasparagine	Dnmasn	N-cycloundecylglycine	Ncund
25	D-N-methylaspartate	Dnmasp	N-(2,2-diphenylethyl)glycine	Nbhm
	D-N-methylcysteine	Dnmcys	N-(3,3-diphenylpropyl)glycine	Nbhe
	D-N-methylglutamine	Dnmgln	N-(3-guanidinopropyl)glycine	Narg
	D-N-methylglutamate	Dnmglu	N-(1-hydroxyethyl)glycine	Nthr
	D-N-methylhistidine	Dnmhis	N-(hydroxyethyl)glycine	Nser
30	D-N-methylisoleucine	Dnmile	N-(imidazolylethyl)glycine	Nhis
	D-N-methylleucine	Dnmleu	N-(3-indolylethyl)glycine	Nhtrp

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	D-N-methyllysine	Dnmlys	N-methyl- $\gamma$ -aminobutyrate	Nmgabu
	N-methylcyclohexylalanine	Nmchexa	D-N-methylmethionine	Dnmmt
	D-N-methylornithine	Dnmorn	N-methylcyclopentylalanine	Nmcpen
	N-methylglycine	Nala	D-N-methylphenylalanine	Dnmphe
5	N-methylaminoisobutyrate	Nmaib	D-N-methylproline	Dnmpro
	N-(1-methylpropyl)glycine	Nile	D-N-methylserine	Dnmser
	N-(2-methylpropyl)glycine	Nleu	D-N-methylthreonine	Dnmthr
	D-N-methyltryptophan	Dnmtrp	N-(1-methylethyl)glycine	Nval
	D-N-methyltyrosine	Dnmtyr	N-methyl- $\alpha$ -naphthylalanine	Nmanap
10	D-N-methylvaline	Dnmval	N-methylpenicillamine	Nmpen
	$\gamma$ -aminobutyric acid	Gabu	N-( <i>p</i> -hydroxyphenyl)glycine	Nhtyr
	L- <i>t</i> -butylglycine	Tbug	N-(thiomethyl)glycine	Ncys
	L-ethylglycine	Etg	penicillamine	Pen
	L-homophenylalanine	Hphe	L- $\alpha$ -methylalanine	Mala
15	L- $\alpha$ -methylarginine	Marg	L- $\alpha$ -methylassparagine	Masn
	L- $\alpha$ -methylasspartate	Masp	L- $\alpha$ -methyl- <i>t</i> -butylglycine	Mtbug
	L- $\alpha$ -methylcysteine	Mcys	L-methylethylglycine	Metg
	L- $\alpha$ -methylglutamine	Mgln	L- $\alpha$ -methylglutamate	Mglu
	L- $\alpha$ -methylhistidine	Mhis	L- $\alpha$ -methylhomophenylalanine	Mhphe
20	L- $\alpha$ -methylisoleucine	Mile	N-(2-methylthioethyl)glycine	Nmet
	L- $\alpha$ -methyllleucine	Mleu	L- $\alpha$ -methyllysine	Mlys
	L- $\alpha$ -methylmethionine	Mmet	L- $\alpha$ -methylnorleucine	Mnle
	L- $\alpha$ -methylnorvaline	Mnva	L- $\alpha$ -methylornithine	Morn
	L- $\alpha$ -methylphenylalanine	Mphe	L- $\alpha$ -methylproline	Mpro
25	L- $\alpha$ -methylserine	Mser	L- $\alpha$ -methylthreonine	Mthr
	L- $\alpha$ -methyltryptophan	Mtrp	L- $\alpha$ -methyltyrosine	Mtyr
	L- $\alpha$ -methylvaline	Mval	L-N-methylhomophenylalanine	Nmhpe
	N-(N-(2,2-diphenylethyl)	Nnbhm	N-(N-(3,3-diphenylpropyl)	Nnbhe
	carbamylmethyl)glycine		carbamylmethyl)glycine	
30	1-carboxy-1-(2,2-diphenyl-Nmbc			
	ethylamino)cyclopropane			

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Crosslinkers can be used, for example, to stabilise 3D conformations, using homo-bifunctional crosslinkers such as the bifunctional imido esters having  $(\text{CH}_2)_n$  spacer groups with  $n=1$  to  $n=6$ , glutaraldehyde, N-hydroxysuccinimide esters and hetero-  
5 bifunctional reagents which usually contain an amino-reactive moiety such as N-hydroxysuccinimide and another group specific-reactive moiety.

Reference to "down-regulating or otherwise reducing" the functional level of an endogenously-produced molecule should be understood as a reference to either partially  
10 or completely reducing the functional level of said molecule. For example, it may be necessary to render non-functional all available cytokine, adhesion molecule or complement component or only a portion thereof, depending on the required outcome. For example, it may be necessary to eliminate or merely reduce the severity of one or more symptoms which result from the availability of the functionally active cytokine,  
15 adhesion molecules or complement component. Further, it should also be understood that it may be desirable to systemically reduce functional levels of, for example, a cytokine or adhesion molecule or complement component or to functionally reduce a cytokine, adhesion molecule or complement component level in a localised region only, such as a region of inflammation (for example, an arthritic joint).

20

Cytokines form part of a complex network wherein the functional activity induced by any one cytokine may include the up or down-regulation of the synthesis of another cytokine. For example, IL-1 and  $\text{TNF}\alpha$  are known to stimulate the production of GM-CSF. Accordingly, with respect to cytokines, a related aspect of the present invention should  
25 be understood to extend to indirectly reducing the functional level of a cytokine by administering an effective amount of another cytokine or cytokine receptor, the functional down-regulation of which leads to down-regulation of the production of the former cytokine. For example, by administering  $\text{TNF}\alpha$  or a receptor or a derivative, homologue, analogue, chemical equivalent or mimetic thereof for the purpose of inducing  
30 an immune response to this cytokine, in addition to decreasing the functional levels of  $\text{TNF}\alpha$ , the production of GM-CSF will also be decreased.

- 20 -

Reference to "inducing, up-regulating or otherwise eliciting an immune response directed to" an endogenously-produced molecule should be understood as a reference to eliciting a humoral and/or T helper cell response to said molecule. Preferably, the immune response comprises the stimulation of a Th2 humoral response to provide help to a B cell response which produces antibodies specifically directed to the molecule or ligand of interest.

The agent is administered in an amount effective to induce an immune response to the molecule or ligand which it comprises. (These molecules are herein collectively referred to as the "antigens"). In this regard, reference to an "effective amount" should be understood as a reference to an amount of agent necessary to at least partly achieve the desired outcome.

Without limiting the present invention to any one theory or mode of action, the desired outcome (being the induction of an immune response to the antigen) is effectively an autoimmune response since the immune response of the subject will comprise the production of antibodies specifically directed to one or more molecules or ligands produced by that individual. Accordingly, the method of the present invention provides the opportunity of blocking the ligand at specific sites. This facilitates the minimisation of systemic adverse effects or toxicological events. The method of the present invention is particularly useful since it permits the production of a short lived immune response as a result of a transient expansion and/or activation of B cells thereby providing the option of a short term autoimmune response directed to one or more of these molecules. This is of use, for example, where the condition being treated is transient (such as a transient inflammatory response due to an allergic reaction) and thereby the alleviation of symptoms associated with that condition is necessary only for a finite length of time. Nevertheless, the method of the present invention should also be understood to extend to the induction of long lived immune responses where it is desirable to reduce functional levels of a given cytokine or adhesion molecule on an ongoing basis. In this regard, the method of the present invention can be utilised to provide a sustained action over a period of *inter alia* up to months after initial dosing, thereby reducing the problem of

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compliance to daily dosing. For example, where an individual is suffering from a chronic condition, such as rheumatoid arthritis, Crohn's disease, Type I diabetes, multiple sclerosis, psoriasis or chronic inflammatory lung disease it may be desirable to systemically reduce levels of GM-CSF or other cytokine for indefinite time periods for the purpose of providing some relief from the symptoms of inflammation associated with this disease.

Still without limiting the present invention to any one theory or mode of action, the method of the present invention provides a target specificity which is not attainable utilising small organic molecules. Further, this specificity thereby facilitates the use of smaller amounts of the active agent. A common problem associated with vaccines is the development of a local inflammatory reaction at the site of injection after repeated dosing. This response is termed the Arthus reaction and results from immune complex formation due to antibodies, generated from previous dosing, cross-linking and precipitating a component of the vaccine construct. To the extent that the method of the present invention utilises a peptide-based antigen, the development of an Arthus reaction will likely be reduced.

The method of the present invention may be directed to inducing an immune response to one particular antigen. However, the method also extends to simultaneously eliciting an immune response to a combination of antigens such as, but not limited to, two or more different cytokines, two or more complement components, one or more cytokines and one or more cytokine receptors, one or more adhesion molecules and one or more adhesion molecule ligands.

25

The method of the present invention is useful in relation to disease conditions, one or more symptoms or causes of which are directly or indirectly due to the production of a particular molecule or a disease condition which otherwise involves unwanted production of molecules such as cytokines, adhesion molecules or complement components. For example, the production of  $\text{TNF}\alpha$  and IL-1 in patients suffering from inflammatory conditions such as rheumatoid arthritis, Crohn's disease, Type I diabetes, multiple

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sclerosis, psoriasis and chronic inflammatory lung disease results in the induction of localised sites of inflammation. Chronic inflammation is also facilitated by the presence of GM-CSF and M-CSF. In other examples, elevated levels of TNF- $\alpha$  are involved in congestive cardiac failure and in Type 2 diabetes, where it is linked to the development  
5 of insulin resistance. Osteoporosis and atherosclerosis are associated with increased M-CSF levels. The unwanted cytokine production, adhesion molecule expression or complement activation may be due to the subject's immune response which is naturally induced as a result of the disease condition or the treatment schedule itself. Any unwanted effects, whether occurring naturally due to the immune response or due to  
10 therapeutic treatment, are referred to herein as "unwanted endogenously-produced molecule functional activity".

Accordingly, in another aspect there is provided a method for the treatment or prophylaxis of a disease condition involving unwanted endogenously produced molecule  
15 functional activity in a subject said method comprising administering to said subject an effective amount of an agent, the agent comprising said molecule and/or molecule ligand or a derivative, homologue, analogue, chemical equivalent or mimetic thereof for a time and under conditions sufficient to induce, up-regulate or otherwise elicit an immune response directed to said molecule and/or molecule ligand wherein the functional level of  
20 said molecule is down-regulated.

Preferably, said molecule is a cytokine, adhesion molecule or complement component.

In a particular embodiment, said disease condition is a chronic inflammatory disease  
25 condition and even more particularly rheumatoid arthritis, Type I diabetes, multiple sclerosis, psoriasis or chronic inflammatory lung disease such as asthma, chronic bronchitis, emphysema or chronic obstructive airway disease.

In another particular embodiment, said disease condition is a non-inflammatory disease  
30 condition and even more particularly congestive heart failure, osteoporosis, atherosclerosis or Type 2 diabetes.



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It should be understood that it may be desirable to apply the method of the present invention to prevent the onset of symptoms due to unwanted endogenous molecule production or activity. For example, where it is predicted that unwanted cytokine production or adhesion molecule expression is likely to occur, the method of the present invention may be utilised prophylactically to prevent the initiation of any symptoms.

The agent which is administered in accordance with the method of the present invention preferably comprises specific immunogenic peptide regions of the subject antigen to which it is desired to elicit a specific immune response. For example, with respect to a cytokine, the peptide region may be the region which interacts with the cytokine receptor. With respect to a cytokine receptor, the peptide region may be the region which interacts with the cytokine. Any given agent may comprise two or more peptide regions from the same cytokine, cytokine receptor, adhesion molecule or adhesion molecule ligand. This would facilitate, for example, the induction of an immune response to more than one site on the subject antigen. The agent may also comprise two or more peptide regions from different cytokines, cytokine receptors, adhesion molecules or adhesion molecule ligands. The latter agents would facilitate the elicitation of immune responses directed to two or more cytokines, cytokine receptors, adhesion molecule or adhesion molecule ligands.

Without limiting the scope or operation of the present invention in any way, particularly preferred agents for use in the method of the present invention comprise at least one B cell epitope and at least one T cell epitope. The T cell epitope is designed to stimulate a Th2 humoral response which provides "help" in relation to the antibody response which is generated to the one or more B cell epitopes which comprise the agent.

25

Reference to a "B cell epitope" should be understood as a reference to any molecule to which an antibody may bind. It should be understood that although reference to "B cell epitope" is a reference to a molecule to which an antibody can bind, it is not intended to be limited to only that part of the molecule to which the antibody actually binds.

Accordingly, the B cell epitope may be a larger molecule which comprises a discrete antibody binding region. In accordance with the present invention, the B cell epitope

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may also be a small peptide the entirety of which comprises the antibody binding site. Where the B cell epitope is a larger molecule, it may comprise, for example, all or part of one chain of a cytokine. This chain may inherently comprise more than one antibody binding site. Accordingly, the epitopes which comprise the agents used in the present invention may be separate molecules or they may comprise discrete regions of a single larger molecule. The epitope may be immunogenic in its own right or it may require coupling to a carrier molecule and/or administration together with an adjuvant and/or a co-adjuvant to stimulate a humoral immune response.

Reference to a "T cell epitope" should be understood as a reference to a molecule which, following antigen presenting cell processing and MHC Class II expression will induce a T-helper cell response. Preferably, the T-helper cell response is a Th2 humoral response which supports the induction of a humoral immune response. Whereas the B cell epitope must be selected from the antigen to which the humoral immune response is to be directed, this is not necessarily the case with the T cell epitope. The T cell epitope may be any molecule which will stimulate a Th2 humoral immune response such as, but not limited to, viral proteins, Ovalbumin 323-339 [Kjerrulf M., 1997], F protein of measles 288-302 [Partidos C., 1992], T cell epitope (P30) tetanus toxoid [Astori M., 1996], Haemagglutinin (H) of measles virus peptide 39 [Obeid O., 1993], E-7 protein of human papillomavirus (AHYNIVTFCKK) [ $<400>1$ ] [Vandebriel R., 1995], immunodominant T cell epitope on cholera toxin [Cong Y., 1996] and peptides from influenza haemagglutinin. Nevertheless the T cell epitope may comprise part of the subject antigen. It should be understood that the T cell epitope may be larger than the peptide which is ultimately processed and expression in the MHC Class II cleft. In fact, a single molecule may, upon processing, give rise to two or more different peptides which are expressed by the antigen presenting cells.

In one embodiment of the present invention, the B cell epitopes and T cell epitopes are selected such as to minimise the polyvalency of the antigen. Without limiting the present invention to any one theory or mode of action, this will reduce the number of antibodies that bind to the antigen thereby maintaining the solubility of the antigen-antibody

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complex. This minimises the risk of developing an inflammatory immune response to insoluble complexes (i.e. the Arthus reaction). This is of use, *inter alia*, where the method of the present invention is utilised in the treatment of chronic diseases where prolonged treatment requiring repeated dosing of the antigen may be required.

5

The B cell epitope and the T cell epitope may be linked, bound or otherwise associated. This association may be direct whereby the B cell epitope is linked to the T cell epitope via any suitable mechanism such as covalent bonds, ionic interaction, electrostatic interaction or Van der Waals Forces. Alternatively, the B cell epitope and T cell epitope  
10 may be indirectly linked via an unrelated proteinaceous or non-proteinaceous molecule.

The B and T cell epitopes may be administered in isolation or together with an adjuvant or other immunomodulatory molecule. Adjuvants suitable for use in the present invention are well known to the person skilled in the art. However, examples of suitable  
15 adjuvants include, but are not limited to, MS59 (Chiron), Montanide ISA 720 (SEPPIC, France), Quilimmune (Aquila Biopharmaceutical), aluminium salts, colloidal iron hydroxide, incomplete Freund's, Co- adjuvants are agents included to enhance the immune response and examples include GM-CSF, Quil A, LPS, muramyl dipeptide.

20 Accordingly, in another aspect the present invention provides an immunogenic agent for use in inducing, up-regulating or otherwise eliciting an immune response to an endogenously-produced molecule or ligand thereof, said composition comprising at least one B cell epitope linked, bound or otherwise associated with at least one T cell epitope.

25 More particularly, the present invention provides an immunogenic agent for use in inducing, up-regulating or otherwise eliciting an immune response to an endogenously-produced molecule or ligand thereof said composition comprising at least two B cell epitopes which epitopes are each linked, bound or otherwise associated with at least three T cell epitopes.

30

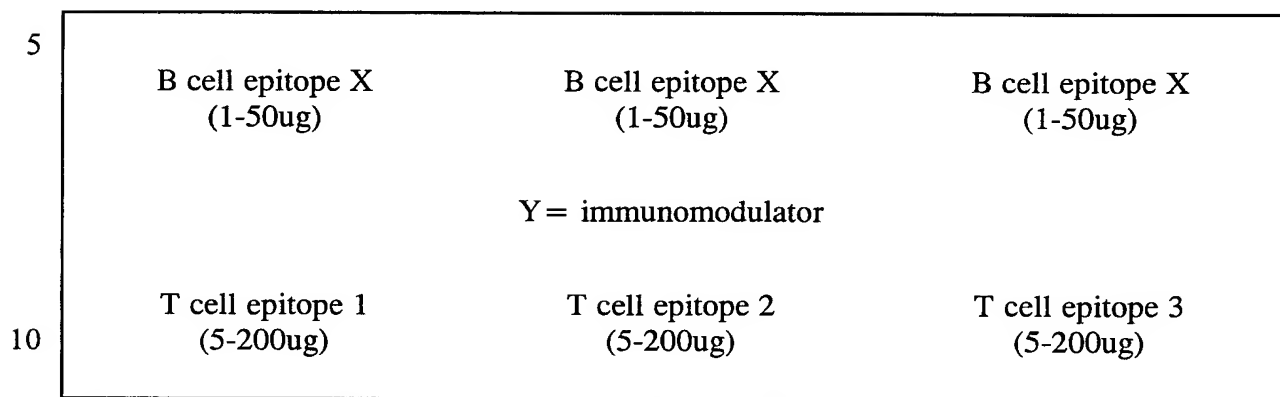
Preferably, said molecule is a complement component, cytokine or adhesion molecule.

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Even more preferably said cytokine is M-CSF, GM-CSF and/or  $\text{TNF}\alpha$  and said adhesion molecule is Integrin  $\alpha_4\beta_1$ .

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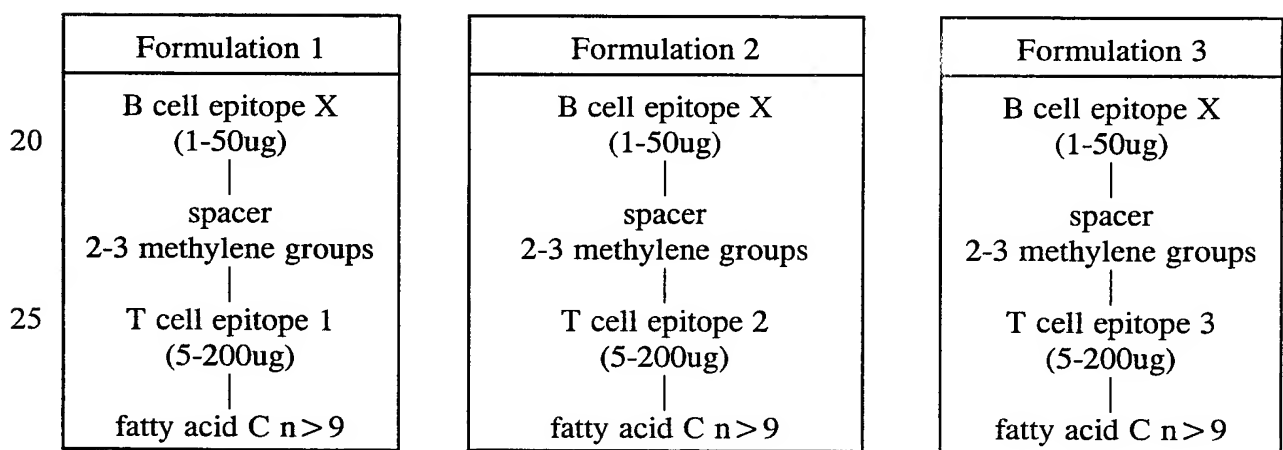
Particularly preferred immunogenic agents according to these aspects of the present invention comprise the structure:



Water in oil emulsion Montanide ISA 720

15

or



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Yet another aspect of the present invention provides a pharmaceutical composition for use in down-regulating the functional activity of an endogenously-produced molecule comprising an agent as hereinbefore defined together with any one or more pharmaceutically acceptable carriers and/or diluents. These components are referred to  
5 as the active ingredients.

Administration of the immunogenic agent, in the form of a pharmaceutical composition, may be performed by any convenient means. The immunogenic agent is contemplated to exhibit therapeutic activity when administered in an amount which depends on the  
10 particular case. The variation depends, for example, on the human or animal and the agent chosen. A broad range of doses may be applicable. Considering a patient, for example, from about 1.0µg to about 1mg of agent may be administered per dosing, and more preferably 1.0µg to about 50µg per dosing. Dosage regimes may be adjusted to provide the optimum therapeutic response. For example, doses may be administered  
15 weekly, monthly or other suitable time intervals or the dose may be proportionally reduced as indicated by the exigencies of the situation. The agent may be administered in a convenient manner such as by the oral, intravenous (where water soluble), intranasal, intraperitoneal, intramuscular, subcutaneous, intradermal or suppository routes or implanting (e.g. using slow release molecules). With particular reference to use of the  
20 agent, these agents may be administered in the form of pharmaceutically acceptable nontoxic salts, such as acid addition salts or metal complexes, e.g. with zinc, iron or the like (which are considered as salts for purposes of this application). Illustrative of such acid addition salts are hydrochloride, hydrobromide, sulphate, phosphate, maleate, acetate, citrate, benzoate, succinate, malate, ascorbate, tartrate and the like. If the active  
25 ingredient is to be administered in tablet form, the tablet may contain a binder such as tragacanth, corn starch or gelatin; a disintegrating agent, such as alginic acid; and a lubricant, such as magnesium stearate.

Routes of administration include but are not limited to intravenously, intraperitoneal,  
30 subcutaneously, intracranial, intradermal, intramuscular, intraocular, intrathecal, intracerebrally, intranasally, infusion, orally, rectally, *via* iv drip, patch and implant.

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Intravenous routes are particularly preferred.

Compositions suitable for injectable use include sterile aqueous solutions (where water soluble) and/or emulsions or liposome preparations or sterile powders for the  
5 extemporaneous preparation of sterile injectable solutions. They must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol and liquid polyethylene glycol, and the like), suitable mixtures  
10 thereof and vegetable oils. The preventions of the action of microorganisms can be brought about by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, thimerosal and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars or sodium chloride. Prolonged absorption of the injectable compositions can be brought about by the use in the  
15 compositions of agents delaying absorption, for example, aluminum monostearate and gelatin or encapsulation in a biocompatible polymer such as PLA polylactic and/or PLG polyglycolic acid.

Sterile injectable solutions are prepared by incorporating the active compounds in the  
20 required amount in the appropriate solvent with various of the other ingredients enumerated above, as required, followed by, for example, filter sterilization or sterilization by other appropriate means. Dispersions are also contemplated and these may be prepared by incorporating the various sterilized active ingredients into a sterile vehicle which contains the basic dispersion medium and the required other ingredients  
25 from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, a preferred method of preparation includes vacuum drying and the freeze-drying technique which yield a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution.

30 When the active ingredients are suitably protected, they may be orally administered, for example, with an inert diluent or with an assimilable edible carrier, or it may be enclosed

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in hard or soft shell gelatin capsule, or it may be compressed into tablets. For oral therapeutic administration, the active compound may be incorporated with excipients and used in the form of ingestible tablets, buccal tablets, troches, capsules, elixirs, suspensions, syrups, wafers, and the like. The percentage of the compositions and  
5 preparations may, of course, be varied and may conveniently be between about 5 to about 80% of the weight of the unit. The amount of active compound in such therapeutically useful compositions such that a suitable dosage will be obtained. Preferred compositions or preparations according to the present invention are prepared so that an oral dosage unit form contains between about 0.1mg and 2000 mg of active compound.

10

The tablets, troches, pills, capsules and the like may also contain the components as listed hereafter: A binder such as gum, acacia, corn starch or gelatin; excipients such as dicalcium phosphate; a disintegrating agent such as corn starch, potato starch, alginic acid and the like; a lubricant such as magnesium stearate; and a sweetening agent such a  
15 sucrose, lactose or saccharin may be added or a flavouring agent such as peppermint, oil of wintergreen, or cherry flavouring. When the dosage unit form is a capsule, it may contain, in addition to materials of the above type, a liquid carrier. Various other materials may be present as coatings or to otherwise modify the physical form of the dosage unit. For instance, tablets, pills, or capsules may be coated with shellac, sugar or  
20 both. A syrup or elixir may contain the active compound, sucrose as a sweetening agent, methyl and propylparabens as preservatives, a dye and flavouring such as cherry or orange flavour. Any material used in preparing any dosage unit form should be pharmaceutically pure and substantially non-toxic in the amounts employed. In addition, the active compound(s) may be incorporated into sustained-release preparations and  
25 formulations.

The present invention also extends to forms suitable for topical application such as creams, lotions and gels.

30 Pharmaceutically acceptable carriers and/or diluents include any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption



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delaying agents and the like. The use of such media and agents for pharmaceutically active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active ingredient, use thereof in the therapeutic compositions is contemplated. Supplementary active ingredients can also be incorporated  
5 into the compositions.

It is especially advantageous to formulate parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the mammalian subjects to be  
10 treated; each unit containing a predetermined quantity of active material calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the novel dosage unit forms of the invention are dictated by and directly dependent on (a) the unique characteristics of the active material and the particular therapeutic effect to be achieved and (b) the limitations inherent in the art of  
15 compounding such an active material.

Effective amounts of the composition contemplated by the present invention will vary depending on the severity of the pain and the health and age of the recipient. In general terms, effective amounts may vary from 0.01 ng/kg body weight to about 1 mg/kg body  
20 weight and preferably 0.01 ng/kg body weight to about 1 µg/kg body weight. Alternative amounts include for about 0.1 ng/kg body weight about 10 µg/kg body weight or from 1.0 ng/kg body weight to about 80 µg/kg body weight.

The pharmaceutical composition may also comprise genetic molecules such as a vector  
25 capable of transfecting target cells where the vector carries a nucleic acid molecule or derivative or analogue thereof capable of expressing an endogenously produced molecule or derivative, homologue, analogue or mimetic thereof. The vector may, for example, be a viral vector.

30 The agent may also be linked to a targeting means such as monoclonal antibody, which provides specific delivery of the agent to a target region.

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In accordance with a method of the invention, the immunogenic agent may be coadministered with one or more other compounds or molecules. For example, the agent may be coadministered with an unrelated cytokine, such as cytokine which up-regulates a humoral and/or Th2 humoral immune response. In another example, where the patient is  
5 suffering from an inflammatory condition, the agent may be coadministered with another molecule designed to reduce or alleviate any one or more symptoms of the inflammatory response. By "coadministered" is meant simultaneous administration in the same formulation or in two different formulations via the same or different routes or sequential administration by the same or different routes. By "sequential administration" is meant a  
10 time difference of from seconds, minutes, hours or days between the administration of the two types of molecules. These molecules may be administered in any order.

Yet another aspect of the present invention is directed to an immunogenic agent as hereinbefore defined when used in accordance with the method of the present invention.  
15

Still another aspect of the present invention provides an agent useful for down-regulating the functional level of an endogenously-produced molecule in a subject as hereinbefore defined.

20 Still yet another aspect of the present invention provides an immunogenic agent in the manufacture of a medicament for the treatment of a disease condition characterised by unwanted endogenously produced molecule levels.

In a particular embodiment, said disease condition is chronic inflammatory disease and  
25 even more particularly rheumatoid arthritis, Crohn's disease, Type I diabetes, multiple sclerosis, psoriasis or chronic inflammatory lung disease (asthma, chronic bronchitis, emphysema or chronic obstructive airway disease).

The present invention should also be understood to extend to down-regulating or  
30 otherwise reducing the functional level of an endogenously-produced molecule in a subject via *ex vivo* immunisation. *Ex vivo* immunisation is achieved by the *in vitro*

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exposure of autologous dendritic cells and autologous B cells to the B cell epitope and the T cell epitope. Said dendritic cells and B cells undergo *in vitro* colony expansion utilising colony expansion factors and cytokines. The primed colony expanded dendritic cells and B cells are introduced into the subject for a time and under conditions sufficient  
5 to induce, up-regulate or otherwise elicit an immune response to said molecule or ligand thereof.

Further features of the present invention are defined in the following non-limiting Examples.

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### EXAMPLE 1

#### VACCINE FORMULATION

Antigen, X = cytokine and/or same cytokine receptor and/or peptide fragment of  
 5 cytokine and/or same cytokine receptor

Antigen will be at least two or more peptide sequences one or more from the cytokine  
 and one or more from the cytokine receptor.

T cell epitopes will be at least three per peptide with 3-6 per B cell peptide

Adjuvant Water in oil emulsion Montanide ISA 720 (SEPPIC, France) (3 parts saline  
 10 plus 7 parts adjuvnt)

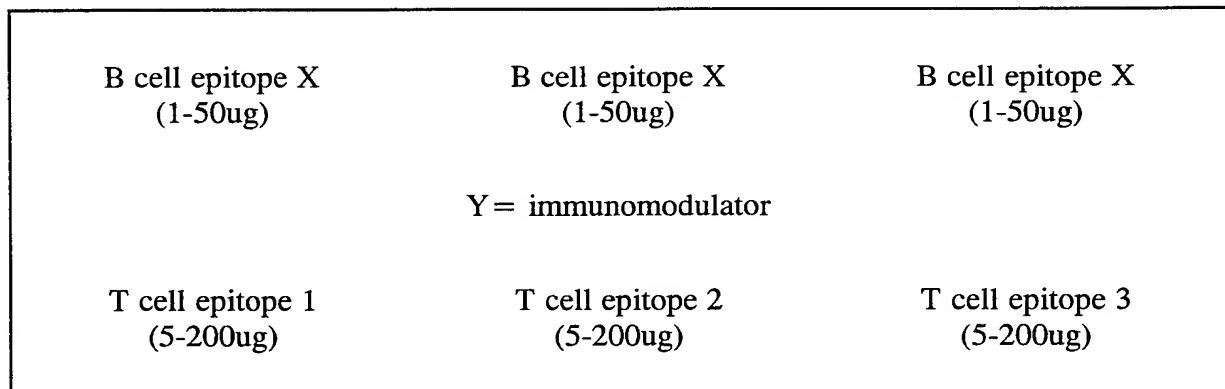
Co-adjuvant GMCSF, Quil A or LPS

Dose 0.5 ml

Vaccine Construct. Components separate in solution

15

20



25 Water in oil emulsion Montanide ISA 720 (SEPPIC, France) (3 parts saline plus 7 parts  
 adjuvant)

X = cytokine and/or receptor and/or peptide fragment of cytokine and/or receptor

Y = GMCSF, Quil A or LPS.

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## EXAMPLE 2

### VACCINE FORMULATION

Antigen, spacer 2-3 methylene groups, T cell epitopes, fatty acid C 10 Oil in water

5 emulsion MS 59 (Chiron). 5% oil in water

Dose 0.5 ml

Antigen is two or more peptide sequences, one or more from the cytokine and one or more from the receptor.

10

T cell epitopes will be at least three per peptide.

Adjuvant. In this example the Chiron adjuvant MS 59

15 Components in each formulation are covalently linked.

Formulation 1	Formulation 2	Formulation 3
<p>20 B cell epitope X (1-50ug)</p> <p>spacer</p> <p>2-3 methylene groups</p> <p>25 T cell epitope 1 (5-200ug)</p> <p>fatty acid C n &gt; 9</p>	<p>B cell epitope X (1-50ug)</p> <p>spacer</p> <p>2-3 methylene groups</p> <p>T cell epitope 2 (5-200ug)</p> <p>fatty acid C n &gt; 9</p>	<p>B cell epitope X (1-50ug)</p> <p>spacer</p> <p>2-3 methylene groups</p> <p>T cell epitope 3 (5-200ug)</p> <p>fatty acid C n &gt; 9</p>

Y = immunomodulator

30

Oil in water emulsion Chiron adjuvant MF 59 (5% oil in water)

B cell epitope X = cytokine and/or receptor and/or peptide fragment of cytokine and/or receptor

35

Y = GMCSF, Quil A or LPS.

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### EXAMPLE 3

#### ANTIGENS

- (i) The antigen is a peptide from the N terminus alpha-helix region of GM-CSF.

5 This region includes residues 14-25 but extends to encompass those residues that can contribute to an antigen that can produce an antibody that blocks the biological activity of GM-CSF. The peptide sequence will be at least six residues and may be linear or cyclised to produce a spatial conformation such as an alpha helix.

10

The second element is a peptide from the GM-CSF receptor including residues around residue 367 of the alpha unit.

- (ii) The antigen is a peptide that includes the residues 106-127 within the fourth helix  
15 near the carboxy terminal tail. This region includes residues 106-127 but extends to encompass those residues that can contribute to an antigen that can produce an antibody that blocks the biological activity of GM-CSF.

The peptide sequence is at least six residues and may be linear or cyclised to  
20 produce a spatial conformation such as an alpha helix.

The second element is a peptide from the GM-CSF receptor including residues around residue 280 of the alpha unit.

- 25 (iii) This antigen is a peptide that includes residues 78-94 in the middle of the third alpha helix.

This region includes residues 78-94 but extends to encompass those residues that can contribute to an antigen that can produce an antibody that blocks the  
30 biological activity of GM-CSF.

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- (iv) The antigen is a peptide from the N terminus alpha-helix region of M-CSF. This region includes residues 9, 15 and 19 but extends to encompass those residues that can contribute to an antigen that can produce an antibody that blocks the biological activity of M-CSF.

5

The peptide sequence is at least six residues and may be linear or cyclised to produce a spatial conformation such as an alpha helix.

10

The second element is a peptide from the M-CSF receptor including residues within the three N-terminal immunoglobulin-like domains.

15

- (v) The antigen is a peptide from the N terminus alpha-helix region of TNF. This region includes residues 32-42, 81-88 and 133-137 but extends to encompass those residues that can contribute to an antigen that can produce an antibody that blocks the biological activity of TNF.

The peptide sequence is at least six residues and may be linear or cyclised to produce a spatial conformation such as an alpha helix.

20

The second element is a peptide from the TNF receptor including residues 116-124, fourth cysteine rich domain 143-160, N-terminal extracellular domain 159-178 but extending to encompass those residues that can contribute to an antigen that can produce an antibody that blocks the biological activity of TNF.

25 (vi)

The antigen is a peptide from the beta-turn structure of the third N-terminal repeat in alpha-4 of integrin alpha4-beta1. This region includes residues 181-190 (GAPGSSYWTG [ $<400>2$ ]) but extends to encompass those residues that can contribute to an antigen that can produce an antibody that blocks the biological activity of integrin alpha4-beta1.

30

The peptide sequence is at least six residues and may be linear or cyclised to

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produce a spatial conformation such as an alpha helix.

The second element is a peptide from the VCAM receptor including residues 36-43 but extending to encompass those residues that can contribute to an antigen that  
5 can produce an antibody that blocks the biological activity of integrin alpha4-beta1.

Those skilled in the art will appreciate that the invention described herein is susceptible to variations and modifications other than those specifically described. It is to be  
10 understood that the invention includes all such variations and modifications. The invention also includes all of the steps, features, compositions and compounds referred to or indicated in this specification, individually or collectively, and any and all combinations of any two or more of said steps or features.



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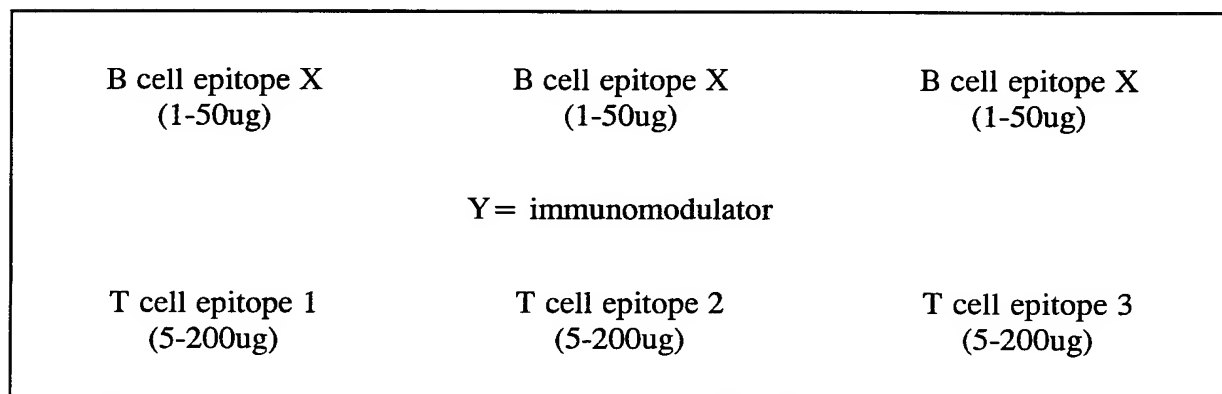
- 40 -

CLAIMS:

1. A method of down regulating or otherwise reducing the functional level of an endogenously-produced molecule in a subject said method comprising administering to said subject an effective amount of an agent, the agent comprising said molecule or the ligand of said molecule or a derivative, homologue, analogue, chemical equivalent or mimetic thereof for a time and under conditions sufficient to induce, upregulate or otherwise elicit an immune response directed to said molecule and/or said ligand.
2. The method according to claim 1 wherein said endogenously produced molecule is a complement component, cytokine or adhesion molecule.
3. The method according to claim 2 wherein said complement component is C1q, C1r, C1s, C3, C4, Factor D, plasma protease, Factor B, C2b, C2a, C3b, C3bBb, C4b2b or the membrane attack complex.
4. The method according to claim 2 wherein said cytokine is GM-CSF, M-CSF or TNF- $\alpha$  and said agent comprises the subject cytokine or the subject cytokine receptor.
5. The method according to claim 2 wherein said adhesion molecule is Integrin  $\alpha_4 \beta_1$  and said agent comprises Integrin  $\alpha_4 \beta_1$ , VCAM-1 and/or fibronectin.
6. The method according to any one of claims 1-5 wherein said agent comprises at least one B cell epitope linked, bound or otherwise associated with at least one T cell epitope.
7. The method according to claim 6 wherein said agent comprises at least two B cell epitopes which are each linked, bound or otherwise associated with at least 3 T cell epitopes.

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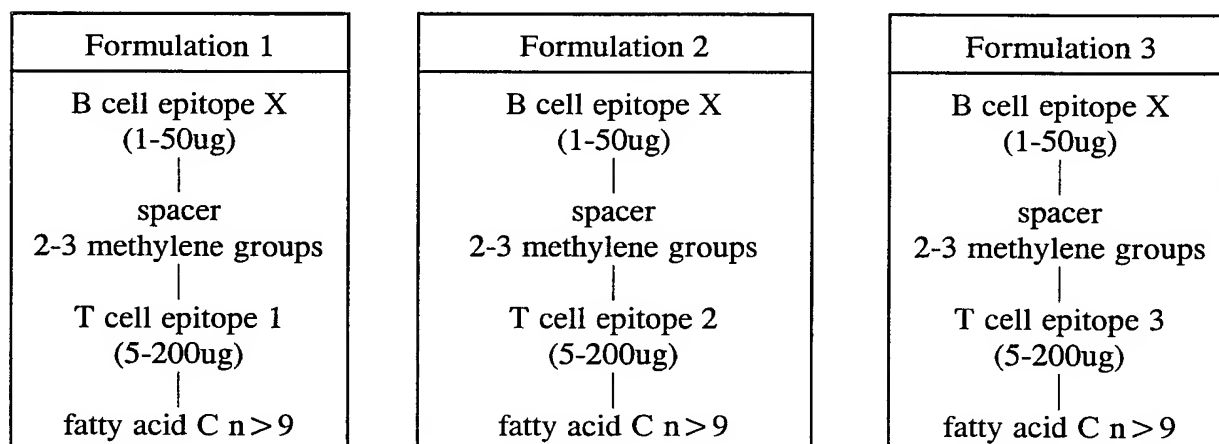
8. The method according to claim 6 or 7 wherein said agent comprises the structure:



|

Water in oil emulsion Montanide ISA 720

or



9. A method for the treatment or prophylaxis of a disease condition involving unwanted endogenously produced molecule functional activity in a subject said method comprising administering to said subject an effective amount of an agent, the agent comprising said molecule and/or molecule ligand or a derivative, homologue, analogue, chemical equivalent or mimetic thereof for a time and under conditions sufficient to

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induce, up-regulate or otherwise elicit an immune response directed to said molecule and/or molecule ligand wherein the functional level of said molecule is down-regulated.

10. The method according to claim 9 wherein said endogenously produced molecule is a complement component, cytokine or adhesion molecule.

11. The method according to claim 10 wherein said complement component is C1q, C1r, C1s, C3, C4, Factor D, plasma protease, Factor B, C2b, C2a, C3b, C3bBb, C4b2b or the membrane attack complex.

12. The method according to claim 10 wherein said cytokine is GM-CSF, M-CSF or TNF- $\alpha$  and said agent comprises the subject cytokine or the subject cytokine receptor.

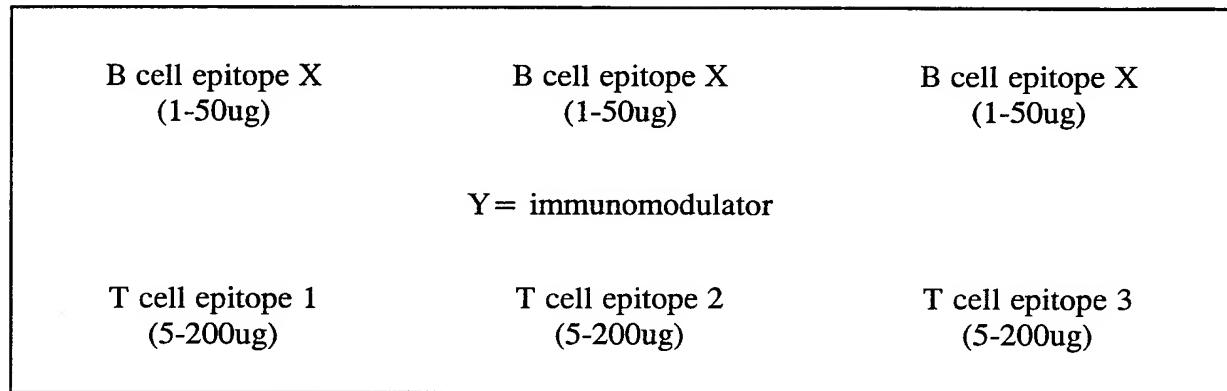
13. The method according to claim 10 wherein said adhesion molecule is Integrin  $\alpha_4\beta_1$  and said agent comprises Integrin  $\alpha_4\beta_1$ , VCAM-1 and/or fibronectin.

14. The method according to any one of claims 9-13 wherein said agent comprises at least one B cell epitope linked, bound or otherwise associated with at least one T cell epitope.

15. The method according to claim 14 wherein said agent comprises at least two B cell epitopes which are each linked, bound or otherwise associated with at least 3 T cell epitopes.

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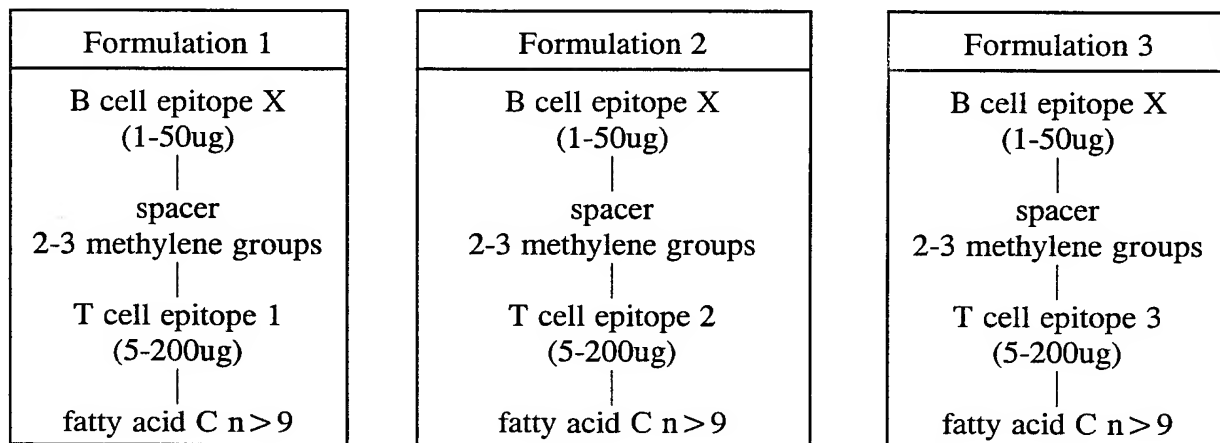
16. The method according to claim 14 or 15 wherein said agent comprises the structure:



|

Water in oil emulsion Montanide ISA 720

or



17. The method according to claim 9 wherein said molecule is a cytokine, adhesion molecule or complement component and said disease condition is a chronic inflammatory disease condition or a non-inflammatory disease condition.

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18. The method according to claim 17 wherein said chronic inflammatory disease condition is rheumatoid arthritis, Type I diabetes, multiple sclerosis, psoriasis or chronic inflammatory lung disease and said non-inflammatory disease condition is congestive heart failure, osteoporosis, atherosclerosis or Type 2 diabetes.

19. An immunogenic agent for use in inducing, up-regulating or otherwise eliciting an immune response to an endogenously-produced molecule or ligand thereof, said agent comprising said molecule or the ligand of said molecule or a derivative, homologue, analogue, chemical equivalent or mimetic thereof.

20. The immunogenic agent according to claim 19 wherein said molecule is a complement component, cytokine or adhesion molecule.

21. The immunogenic agent according to claim 19 wherein said complement component is C1q, C1r, C1s, C3, C4, Factor D, plasma protease, Factor B, C2b, C2a, C3b, C3bBb, C4b2b or the membrane attack complex.

22. The immunogenic agent according to claim 19 wherein said cytokine is GM-CSF, M-CSF or TNF- $\alpha$  and said agent comprises the subject cytokine or the subject cytokine receptor.

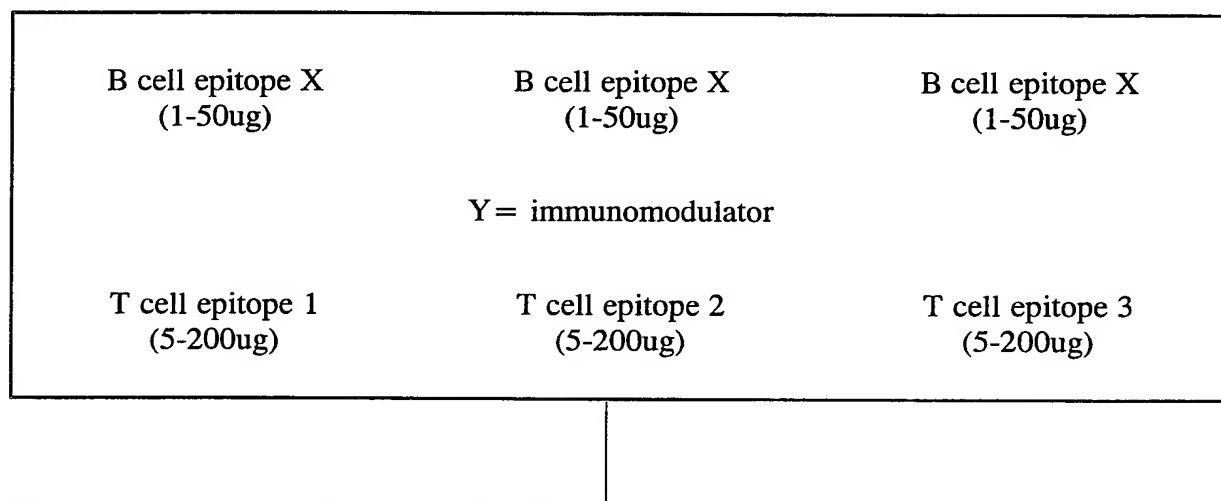
23. The immunogenic agent according to claim 19 wherein said adhesion molecule is Integrin  $\alpha_4 \beta_1$  and said agent comprises Integrin  $\alpha_4 \beta_1$ , VCAM-1 and/or fibronectin.

24. The immunogenic agent according to any one of claims 19-23 wherein said agent comprises at least one B cell epitope linked, bound or otherwise associated with at least one T cell epitope.

25. The immunogenic agent according to claim 24 wherein said agent comprises at least two B cell epitopes which are each linked, bound or otherwise associated with at least 3 T cell epitopes.

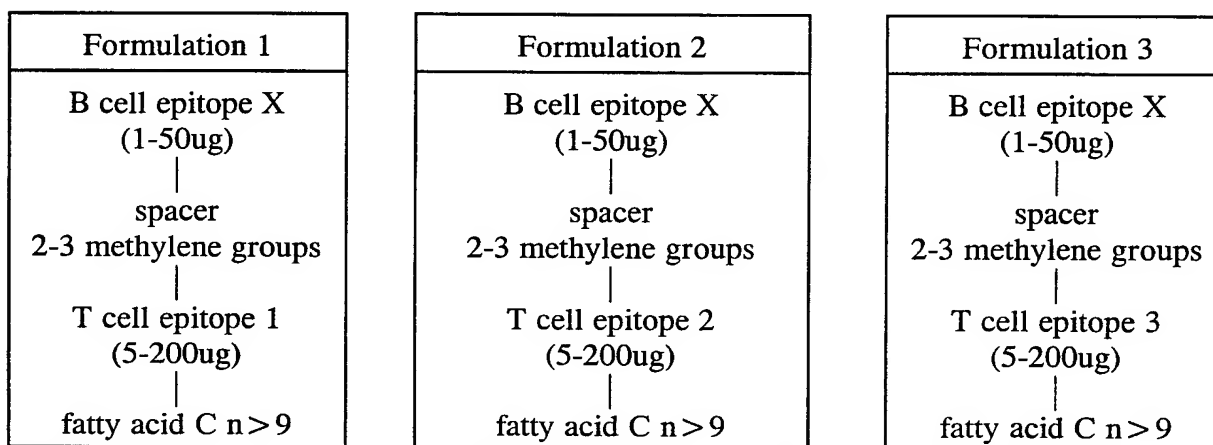
- 45 -

26. The immunogenic agent according to claim 24 or 25 wherein said agent comprises the structure:



Water in oil emulsion Montanide ISA 720

or



27. An immunogenic agent according to any one of claims 19-26 when used in accordance with the method of any one of claims 1-18.

28. Use of an immunogenic agent according to any one of claims 19-26 in the manufacture of a medicament for the treatment of a condition as defined in any one of

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claims 1-18.

29. An agent according to any one of claims 19-26 useful for down-regulating the functional level of an endogenously produced molecule as defined in any one of claims 1-18.

30. A pharmaceutical composition for use in down-regulating the functional activity of an endogenously produced molecule said composition comprising an agent according to any one of claims 19-26 together with any one or more pharmaceutically acceptable carriers and/or diluents.



- 1 -

## SEQUENCE LISTING

&lt;110&gt; BIOFAN PTY LTD

&lt;120&gt; A METHOD OF PROPHYLAXIS AND TREATMENT AND AGENTS USEFUL THEREFOR

&lt;130&gt; 2176520/EJH/TDO/LMa

&lt;140&gt;

&lt;141&gt;

&lt;160&gt; 2

&lt;170&gt; PatentIn Ver. 2.0

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&lt;212&gt; PRT

&lt;213&gt; mammalian

&lt;400&gt; 2

Gly Ala Pro Gly Ser Ser Tyr Trp Thr Gly

1 5 10

## INTERNATIONAL SEARCH REPORT

International application No.  
**PCT/AU00/00424****A. CLASSIFICATION OF SUBJECT MATTER**Int. Cl. <sup>7</sup>: A61K 38/19, 38/48, 38/36, 38/17; A61P 29/00, 25/28, 37/02

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

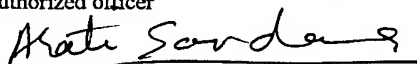
Minimum documentation searched (classification system followed by classification symbols)

**FILE WPAT AND CHEM ABS. SEE DETAILS IN ELECTRONIC DATABASE BASE BELOW**Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched  
**FILE MEDLINE**Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)  
DERWENT WPAT, CHEM ABS, MEDLINE KEYWORDS: TNF $\alpha$ , GM-GSF, M-CSF, Adhesion molecule, Integrin, VCAM, B cell epitope, Fibronectin, Cytokine, Plasma Protease, Factor D, T cell epitope**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 98/46642 A (FARMACEUTISK LABORATORIUM FERRING A/S) 22 October 1998 See whole document.	1-30
X	WO 95/05849 A (MOURITSEN & ELSNER A/S) 2 March 1995 See whole document.	1-30
X	WO 95/11253 A (THE WALTER AND ELIZA HALL INSTITUTE OF MEDICAL RESEARCH) 27 April 1995 See whole document.	1-30

☒ Further documents are listed in the continuation of Box C ☒ See patent family annex

* Special categories of cited documents:	
"A" document defining the general state of the art which is not considered to be of particular relevance	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"E" earlier application or patent but published on or after the international filing date	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"O" document referring to an oral disclosure, use, exhibition or other means	"&" document member of the same patent family
"P" document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search <b>18 July 2000</b>	Date of mailing of the international search report <b>19 JUL 2000</b>
Name and mailing address of the ISA/AU AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA E-mail address: pct@ipaustalia.gov.au Facsimile No. (02) 6285 3929	Authorized officer  <b>ARATI SARDANA</b> Telephone No : (02) 6283 2627

## INTERNATIONAL SEARCH REPORT

International application No.

PCT/AU00/00424

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages (Remove spaces when completed if the page is too long)	Relevant to claim No.
P,X	WO 00/15807 A ((M & E BIOTECH A/S) 23 MARCH 2000 See whole document.	1-30
X	Guidebook to Cytokines and their receptors Edited by Nicos A Nicola. A Sambrook & Tooze Publication pp 103-104 (Aggarwal B.B. and Reddy S. See whole document.	19, 20 and 22
X	Guidebook to Cytokines and their receptors Edited by Nicos A Nicola. A Sambrook & Tooze Publication pp 171-173 (Nicola N.A) See whole document.	19, 20 and 22
X	Guidebook to Cytokines and their receptors Edited by Nicos A Nicola. A Sambrook & Tooze Publication pp 164-167 (Stanley E.R.) See whole document.	19, 20 and 22

## INTERNATIONAL SEARCH REPORT

International application No.

PCT/AU00/00424

**Box I** Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos :  
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☒ Claims Nos : 1, 2, 9, 19 and 20  
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:  
The scope of the above claims is by far too broad and includes a range of structurally and functionally distinct molecules and methods using such molecules. Parts of the above claims referring to derivatives, homologues, analogues, chemical equivalents or mimetics were found to be unsearchable because they are indeterminate in scope.
3. ☐ Claims Nos :  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a)

**Box II** Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

**Remark on Protest**

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

**INTERNATIONAL SEARCH REPORT**  
Information on patent family members

International application No.  
**PCT/AU00/00424**

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report		Patent Family Member (To put a line under the citations tab to the first point on the next row and press F8)					
WO	9846642	AU	70303/98	EP	975668	HR	980203
		NO	995002				
WO	9505849	AU	76080/94	AU	70091/98	CA	2170236
		EP	752886				
WO	9511253	AU	79843/94	CA	2174425	EP	724589
		US	5837694				
							END OF ANNEX

*(To add more lines press TAB at end of last row, remove paragraph marker to join up 'END OF ANNEX' box)*